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BY MARSCHELLE HARNLY POWER

A THESIS

PRESENTED TO THE FACULTY OF
THE GRADUATE COLLEGE OF THE UNIVERSITY OF NEBRASKA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
DEPARTMENT OF CHEMISTRY

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THE OXIDATION OF d-GLUCOSE IN CALCIUM HYDROXIDE SOLUTION BY AIR

INTRODUCTION

The conversion of an aldose into another aldose and several ketoses by weak alkalies or certain salts, first observed by Lobry de Bruyn [1] and later investigated by Nef,[2] was interpreted by the latter as depending on the presence of several "enols," similar to the enol common to glucose, mannose, and fructose suggested by Wohl.[3] In a series studied by Nef and his students on the products obtained, (a) by the action of strong alkalies alone on the sugars, and (b) by the action of strong alkalies plus oxidizing agents, this conception of intermediate enolization is applied, especially in interpreting the results of the oxidation experiments.[4] None of the investigations on oxidation products have, however, been carried out at those weak alkalinities which cause mainly only interconversion, accompanied by saccharinic acid formation, even after considerable time. The purpose of the present work, therefore, was to study such a case. Limewater containing excess suspended calcium hydroxide was selected as approximately fulfilling the desired conditions, as well as affording a convenient means of maintaining a fairly constant hydroxyl ion concentration. It has furnished the alkaline medium for many interconversion experiments,[5] but an air oxidation under such mild and controlled alkaline conditions has not been attempted. Sugar was found to disappear more slowly than when oxidized in stronger alkali; and substances characteristic only of alkaline oxidation could be isolated from the final mixture. The formation of saccharinic acids under the conditions of the experiment was not only possible, but quite probable, however, and this undoubtedly added to the difficulty ordinarily met with in the isolation of the true intermediate oxidation products.

While the results of the research require little comment in that they necessitate no additions to Nef's general theory of sugar oxidation, attention may be directed to the quantitative determination of oxalic acid in the oxidation mixture, in addition to CO_2 and HCOOH, heretofore the two substances susceptible of quantitative estimation in this type of study. Should it become possible to determine more precisely the

[1] Rec. de trav. Chim., 14, 204-216 (1895).
[2] Nef, Ann. 403, 206-217; 338-383 (1914).
[3] Wohl, Ber., 33, 3095 (1900).
[4] Nef, Ref. 4, p. 206 et seq; Glattfeld, Am. Chem. Jour. 50, 135-139 (1913).
[5] Contact of sugar with limewater for some months leads to extensive saccharinic acid formation, as in the case of stronger alkalies).

other intermediate oxidation products, a remote possibility at present, more exact relationships between the sugars should then become apparent, and results of definite physiological value might then be forecasted.

EXPERIMENTAL

The Oxidation

To a four liter suspension of 75 g. CaO, free from other metals and containing 0.13% CO_2, were added a solution containing 100 g. of d-glucose, $[\alpha]D^{20} = +52.5°$ (C=10, moisture 0.20%.), and CO_2 free water sufficient to make a volume of approximately 10 liters. This was sufficient base to provide for the presence of an excess throughout the oxidation, and sufficient water to dissolve five-sixths of an equivalent of lime ($1\ Ca(OH)_2 : 2C_6H_{12}O_6$), the resulting alkali concentration being about N/23. The hydrogen potential of such a solution, after some hours air oxidation, was 0.984 volts against a saturated calomel cell of value 0.247, giving a pH of 12.7, a value very close to that calculated for saturated limewater in the absence of glucose. A strong current of CO_2 free air was immediately drawn through the 20-liter flask containing the mixture, and uniformly distributed at the bottom of the flask, through 16 outlets. Samples were taken periodically without disturbing the air stream, and the oxidation stopped only after a sample showing very slight Fehling's reduction was obtained. After 100 hours, oxidation proceeded slowly. The air stream was stopped at the end of 236 hours and the mixture rapidly filtered. The clear alkaline filtrate and washings amounted to 10.57 liters, and the lime residue when dry approximately 100 g. (Some of this residue was accidentally lost). The non-volatile hydroxy acids obtained from each of the above fractions are described below. They had a combined copper reduction corresponding to 5.1 g. glucose,[6] accordingly 95 g. glucose were oxidized, or otherwise disappeared. None of the brown color developed that always accompanies the action of $Ca(OH)_2$ on glucose in the absence of oxygen.

Volatile Acids — CO_2 and HCOOH

Carbon dioxide determined on 1-2 g. samples of the lime residue amounted to 4.87 g. CO_2 per 100 g. sugar oxidized, while 100 g. d-fructose under similar conditions gave 4.28 g., after applying in each case a blank correction determined by aerating a lime suspension the same length of time and in the same apparatus used for oxidation.

[6] .35 g. glucose in 10.57 liters filtrate; 4.77 g. in the lime residue.

Volatile acids, as determined in portions of the 10.57 liter alkaline filtrate, by vacuum distillation, according to Nef [7] and later modifications, as well as by permanganate [8] titration after distillation, were always variable, but within sufficiently close agreement to show that they are mainly only formic acid: NaOH titrations, 23.5 g. calcd. as HCOOH, per 100 g. glucose burned; $KMnO_4$ titrations, 22.8 g. HCOOH per 100 g. sugar. Volatile acids in the insoluble lime residue were nil, as a few drops only of standard alkali were required to neutralize the distillate after removal of calcium as oxalate.

The Non-Volatile Hydroxy Acids

By exact precipitation of the calcium in 10 liters of original alkaline filtrate, and subsequent distillation in vacuo (water pump) at 50-60°, with a final heating at 85°-95° for some time, 45.1 g. (47.7 for total filtrate) of clear, brown, mobile gummy acids, $[\alpha] = +12.5°$ were obtained.[9] In like manner 27.0 g. brown acids, $[\alpha] = +6.1°$, were obtained from 70 g. lime residue. There were thus about 38 g. material in the total lime residue (100 g.), of which 4.77 g. were glucose, as already noted. The total non-volatile acids from 95 g. glucose under the conditions of this oxidation were, therefore, 80.7 g. Each set of acids was investigated separately, the detailed descriptions of Nef [10] being followed in general. Thorough extractions with hot and cold ethyl acetate and hot and cold ether, effected a resolution of the optically active mixture of acids into a number of high and low rotating fractions. Similar fractions were combined when possible, or when judged desirable. The high rotating fractions were usually treated directly with phenylhdrazine for the purpose of removing d-arabonic acid [11] while the low rotating fractions were, depending on their nature and origin, treated with $CaCO_3$ to remove glycollic acid, or converted into brucine salts, for the purpose of finally obtaining the high melting brucine salts of C_3 and C_4 hydroxyacids.[12] Other fractions, obtained by hydrolysis [13] of gummy hydrazides, non-crystalline brucine salts, etc., after further resolution by solvents, yielded more C_5 hydrazide, glycollic acid, etc., according to their nature.

[7] Nef, Ann., 357, 220-221 (1907).
[8] Jones, Am. Chem. Jour., 17, 539 (1895).
[9] Rotations recorded $[\alpha]$ were made by means of a white light through a 3 cm. 3% dichromate filter. Other more exact rotations made with sodium light and Schmidt and Haensch triple field polariscope reading to 0.01° are given in the usual $[\alpha]$bt form.
[10] Nef, Ann. 357, 221-247; Ann. 403, 242-265.
[11] Glattfeld, Am. Chem. Jour. 135, 139, (1915).
[12] Ref. 4, p. 255.
[13] Procedures followed for preparation and hydrolysis of hydrazides, brucine salts, quinine salts, are described by Glattfeld and Hanke, J. Amer. Chem. Soc., 40, 9661 977; 981 (1918).

TABLE I

Fraction Number	Origin	Weight Grams	[α]	Treatment	New Fractions
1	Ethyl Acetate Soluble	36.2	12.8°	Hot Ether	3, 4
2	Ethyl Acetate Insoluble	7.6	16.8	Phenylhydrazine	5
3	From 1: Ether Soluble	19.2	6.2	Cold Ether	8, 9
4	From 1: Ether Insoluble	14.5	25.9	Hot Ether	10, 11
5	From 2: Sol. Hydrazides Hydrolyzed	5.1	4.0	Hot Ether	6, 7
6	From 5: Ether Soluble	1.7	3.2	Brucine	
7	From 5: Ether Insoluble	3.4	5.8	Brucine	
8	From 3: Ether Soluble	9.2	−0.4	Calcium Carbonate	12
9	From 3: Ether Insoluble	10.1	12.8	Phenylhydrazine	
10	From 4: Ether Soluble	6.6	16.5	Phenylhydrazine	
11	From 4: Ether Insoluble	9.1	30.1	Phenylhydrazine	17
12	From 8: Soluble Calcium Salts by Oxalic Acid precipitation	5.7	2.4	Brucine	
13	From 9: Sol. Hydrazides Hydrolyzed	4.1	13.2		
14	From 10: Sol. Hydrazides Hydrolyzed	3.7	1.9	Cold Ether	15, 16
15	From 14: Ether Soluble	1.5	1.4	Brucine	
16	From 14: Ether Insoluble	2.2	4.7	Brucine	
17	From 11: Sol. Hydrazides Hydrolyzed	6.5	7.4	Cold Ether	18, 19
18	From 17: Ether Soluble	1.5	3.8	Brucine	
19	From 17: Ether Insoluble	4.6	8.5	Brucine	

Table I shows in some detail the resolution of the 45.1 g. acids obtained from first filtrate. After deducting sample for Fehling's determinations. 44.6 g. gave by five extractions with boiling ethyl acetate, about 140 cc. each time (under reflux condenser), the soluble and insoluble portions numbered 1 and 2 respectively. Similar ether extractions of fractions 1 gave fractions 3 and 4. The origin of the remaining fractions, and their subsequent treatment, will be apparent from the table. The hydrazides, brucine, and calcium salts, together with analogous products obtained by similar treatment of 27.0 g. acids from 70 g. lime residue, are summarized below:

Crystalline Phenylhydrazides

These salts, obtained by direct addition of excess phenylhydrazine to the acids dissolved in alcohol, were white after washing with absolute alcohol, with the exception of some from the lime residue, which were yellow due to the presence of some d-glucosazone. Crops obtained were as follows:

Source	*Wt. Grams*	*Melting Point, °C*	[α]
Fractions 2	1.01	177	–7.1
Fraction 9	2.34	197	–9.0
Fraction 10	2.16	203	–14.0
Fraction 11	3.70	198	–10.1
Lime Residue Acids	4.27	183	–14.2
Hydrolyzed Brucine Salts, etc	1.21	190–200	

The properties of these hydrazides indicate that they must be mainly d-arabonic phenylhydrazide. The combined crude crystals, 14.7 g., dissolved in hot water and decolorized with a little Eastman's carbon, deposited over night, 7.59 g. pure white crystals, M.P. 210° and $[\alpha]D^{20} + -14.1°$, in 2.0% aqueous solution, properties that agree with those of d-arabonic phenylhydrazide. A dextro-rotating lactone should therefore result on hydrolysis. Such an acid was obtained on hydrolysis of 6.0 g. of a similar hydrazide from a former experiment, M.P. 214° and $[\alpha]D^{20} = -14.2°$; 3.47 g. crude gum resulted, of which 2.75 g. were soluble in ethyl acetate, and gave, after heating in water solution and finally in vacuo on the boiling water bath $[\alpha] = +57.0°$. The specific rotation of pure d-arabonic lactone in 4% solution is +69.7. Attempts to obtain the crystallized lactone, both from this acid, and from acids obtained by hydrolysis of portions of the above 7.59 g. crystals, and from other hydrazides several times recrystallized, were unsuccessful, however. This finally was accomplished only after purification of the acids

by converting into Ca salts and crystallization of pure calcium d-arabonate, from which the pure acid was obtained by exact precipitation of the Ca as oxalate. About 5.0 g. of the characteristic, fine, white needles of calcium d-arabonate were prepared, one sample of which gave the following analysis: 0.2550 g. dried in vacuo over H_2SO_4 gave, on ignition, 0.0312 g. CaO. Calculated for $Ca(C_5H_6O_9)_2 \cdot 5\ H_2O$, CaO, 12.18%
Found CaO 12.23%
For this salt, $[\alpha]D^{22} = -2.58°$ in agreement with Glattfeld, and Spoehr.[14] (in 1 dm. tube, $\alpha = -0.09°$, p=3.45, d=1.011). Precipitation of Ca from 4.58 g. of the arabonate gave 2.75 g. ethyl acetate soluble, pale yellow gum, strongly dextro-rotatory.

A concentrated ethyl acetate solution gave a small crop of crystals on seeding[15] and evaporation, which could not be satisfactorily separated. The 2.75 g. were again taken up in ethyl acetate and ovaporated, crystals again separating, and by very slow further evaporation in vacuum dessicator and rubbing, finally gave 1.75 g. of the dry lactone crystals, M.P. 90°–92°. Analysis: 0.5524 g. in 4.58% aqueous solution, gave in 1 dm. tube $\alpha = +3.24°$; therefore $[\alpha]D^{20} = +69.6°$. The same solution required 37.23 cc. 0.1 N NaOH for neutralization with phenolphthalein: calculated for pure $C_5H_8O_5$, 37.35 cc. The accumulated evidence thus shows that the original 14.7 g. crude hydrazides were chiefly d-arabonic phenylhydrazide.

The Brucine Salts (C_3 and C_4 Hydroxy Acids)

Fractions 15 and 18, Table I, were combined with two other similar low-rotating acids to give 6.53 g. ether soluble gums, which required 23.5 g. of brucine (15). The dry salts obtained, after removing excess brucine by benzol extraction of their water solution, amounted to 31.3 g. Similarly, fractions 7, 12, 16, and 19, total 15.9 g. required 45.6 g. brucine, and gave 68.7 g. brucine salts. Recrystallization of these salts from absolute alcohol and from alcohol containing a little water finally yielded, from 31.3 g. above, 8.97 g. salts melting at 175°–205°, and from 68.7 g. above, 24.9 g. salts melting at 190°–205°. Combined high melting brucine salts 33.9 g., recrystallized from 1 part water and 12 parts alcohol, gave 13.0 g. melting at 208°, and these, recrystallized from 1 part water and 10 parts alcohol, gave only 6.0 grams of a salt possessing the properties of d-erythronic brucine: after

[14] Spoehr, Am. Chem. Jour., 43, 241.
[15] Some d-arabonic acid lactone crystals for seeding were very kindly given to the author by Professor J. W. E Glattfeld.

extracting dry salt with $CHCl_3$ to remove possible traces of free brucin, M.P. was 210° and $[\alpha]_D^{20}$ = –23.7°, i.e., 2.210 g. in 50 cc. H_2O gave α = –1.05°, in a 1 dm tube. Ruff [16] records the melting point and specific rotation of pure d-erythronic brucine as 215° and –23.5°, respectively. More conclusive evidence for the presence of d-erythronic acid was sought in attempt to prepare the pure lactone, $[\alpha]_D^{20}$ = –73.3,[16] by hydrolysis of the high melting brucine salts. For this purpose a total of 13.4 grams were available, yielding 3.2 g. of fairly stiff acids, $[\alpha]_D^{20}$ = –19.1°, after heating some hours in water solution on boiling water bath and subsequent distillation to dryness. No lactone crystals appeared. If the laevo-rotation was due to d-erthyronic acid or its lactone, it is evident that the 3.2 g. acids were contaminated with considerable inactive material or with dextro-rotating acids. Since the gum was rather dark colored, it was therefore next acetylated, extracted with ether, and again hydrolyzed with $Ba(OH)_2$, etc., to remove possible "sugar tar" that might have inhibited crystallization of the lactone.[17] The acetylated acids soluble in ether, 4.1 g. upon hydrolysis gave 2.2 g. acids, still dark brown. These, thru "norite" treatment, gave 1.82 g. yellow, slightly mobile acids, $[\alpha]_D^{20}$ = –14.7, and practically all soluble in 50 cc. cold ethyl acetate, 1.72 g. Heated in aqueous solution in an open dish 10 hours on the boiling water bath, these lost .13 g. which represents probably glycollic acid; the remaining 1.59 g. had $[\alpha]_D^{20}$ = –18.0°.

On further investigation, the above acid gave 4.9 g. strychnine salts, which from absolute alcohol finally gave 1.81 g. crystalline salts, M.P. 160°–165° (M.P. of pure d-erythronic strychnine, 199°). Hydrolysis with $Ba(OH)_2$, etc., gave an absolute alcohol soluble acid of .44 g., $[\alpha]_D^{20}$ = –32.3°. It was impossible, however, to obtain any lactone crystals, nor could a crystalline phenylhydrazide be prepared from the small amount of material remaining. After hydrolysis of the latter, there remained 0.30 g. absolute alcohol soluble acid, $[\alpha]_D^{20}$ = –31.3, the negative rotation of which was probably due to a small amount of d-erythronic lactone.

The soluble, high melting brucine salts were investigated for the presence of l-glyceric acid. A total of 19.8 g. of such salts gave on hydrolysis 3.9 g. acids $[\alpha]$ +5.1°, which after boiling to remove possible glycollic acid, acetylation and hydrolysis of resulting ether soluble acetylated gums and conversion to quinine salts (5.52 g.), finally gave .29 g. of a

[16] Ruff, Ber., 32, 3678 (1899).
[17] Upson, Jour. Am. Chem. Soc., 40, 461 (1911).

crystalline quinine salt from water. It was easily soluble in 7 cc. hot water, therefore not quinine, and gave after evaporation to 3 cc., .11 g., M.P. 174°, and $[\alpha]D^{22} = -120.5$. The fact that it could be obtained crystalline from water solution is taken as the better evidence that it was the characteristic l-glycerinic quinine. The salt made from pure glyceric acid is variously reported [18] as melting from 176 to 183°, and having a specific rotation of –116.9° to –119.9°.

Glycollic Acid

Fraction 8, Table I, gave with $CaCO_3$, 12.8 g. Ca salts. These after solution in water and precipitation with alcohol, yielded 3.45 g. of almost pure calcium glycollate: i.e., .5327 g. air dried substance lost .1416 g. on drying at 115°, and gave .1088 g. CaO on ignition: calculataed for $Ca(C_2H_3O_2)_2.4\ H_2O$, water 27.48% CaO, 29.47% : Found, Water 26.6%, CaO, 27.8. In all, 6.37 g. such calcium salts were obtained, 1.03 g. and 1.89 g., from ether soluble acids hydrolyzed from gummy brucine salts, in addition to the 3.45 g. above. These recrystallized from 100 cc. water gave 3.29 g. air dried pure $Ca(C_2H_3O_2)_2 4H_2O$: 0.4632 g. dried at 120° gave 0.1323 g. H_2O, and on ignition, 0.0917 g. CaO. Therefore, found, water 28.56, CaO, 29.52.

Oxalic Acid

Hydrochloric acid solutions of portions of lime residue from air oxidations of both glucose and fructose, invariably gave the characteristic white precipitate of calcium oxalate when neutralized with ammonia. It could be repeatedly dissolved and reprecipitated, was insoluble in acetic acid, reduced permanganate readily, and accordingly was undoubtedly calcium oxalate, since the calcium salts of the hydroxyacids are all soluble under these conditions. A preparation, .3474 g., obtained from the oxidation of 35 g. of fructose, after three precipitations from hot acetic acid solutions, and drying at 95-100°, gave .1328 g. CaO on ignition: calculated for $CaC_2O_4:H_2O$, 38.40%, found, 38.23%. Permanganate titration of the precipitate from an aliquot of the fructose lime residue, gave 3.2 g. oxalic acid from 35 g. fructose, corresponding to 9.1 g. from 100 g. fructose. In an experiment in which 10 g. glucose were completely oxidized, 8.08 g. of dry lime residue resulted, 1.981 g. of which, reprecipitated twice

[18] (a) Anderson, Am. Chem. Jour. 42, 423 (1909).
(b) Nef, Hedenberg and Glattfeld, Ibid, 39, 1650 (1917).
(c) Glattfeld and Hanke, J. Am. Chem. Soc. 40, 987 (1918).

from hot HCl solution by ammonia, required 23.2 cc. of 0.0957 N $KMnO_4$; calculated to $H_2C_2O_4$, this amounts to 4.1 g. oxalic acid from 100 g. glucose, less than half that produced from a like weight of fructose.

Qualitative experiments with glucose, NaOH, and air produced no oxalic acid. The relatively large amount here reported is considered to be a primary oxidation product, since it has been shown in this laboratory [19] that glycollic acid is not oxidized by air in alkaline solution.

In the older works in the literature oxalic acid has been detected in traces.[20]

SUMMARY

Of 100 g. d-glucose dissolved in 10 liters of saturated limewater, 95 were oxidized by air in 236 hours. The products, on the basis of 100 g. oxidized, were CO_2, 4.87 g., volatile acids (HCOOH), 25.1 g., non-volatile acids, 84.9 g.

Resolution of the non-volatile acids resulted as follows:

14.7 g. crude l-arabonic phenylhydrazide, which gave 1.75 g. of pure d-arabonic lactone.

6.0 g. brucine salt with the characteristics of d-erythronic brucine, which, however, gave only 0.44 g. of laevo-rotating acid after hydrolysis of the previously prepared strychnine salt.

19.8 g. of alcohol soluble C_3 brucine salt, from which were isolated 0.29 g. of water insoluble quinine salt having the properties of quinine l-glycerate.

6.37 g. of calcium glycollate.

4.1 g. of oxalic acid, quantitatively determined in a separate experiment.

The non-volatile fraction thus consisted largely of unidentified hydroxy acids, the C_3 and C_4 occuring in such small amounts that their identification could not be sharp.

A similar oxidation of fructose gave slightly less CO_2, 4.28 g. per 100, but much more oxalic acid, 9.1 g. Similar d-arabonic phenylhydrazides were also obtained from the non-volatile acids, which were not further investigated.

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[19] R. C. Abbott, Unpublished results.

[20] Nef, Ref. 4, p. 254, from galactose, air and NaOH; Nef, Hedenberg and Glattfeld, Ref. 23b, p. 1645, 1.38 g. oxalic from 50 g. l-arabinose oxidized by $Cu(OH)_2$ and KOH at 100; Glattfeld and Hanke, Ref. 23c, p. 992, 0.14 g. CaC_2O_4 from 100 g. maltose hydrate oxidized by H_2O_2 in KOH.